10/517803
PCT/JP03/07333

1[illegible]6.03

REC'D 27 JUN 2003
WIPO PCT

The Patent Office
PATENTS · DESIGNS · COPYRIGHT · TRADE MARKS

CHARTER MARK

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Signed Andrew Gersey

Dated 3 April 2003

PCT/JP 03/07333

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
H
10 JUN 2002
LONDON

The Patent Office

10.06.03

11JUN02 E724741-1 D00192
P01/7700 0.00-0213286.8

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — P N.85898 TAC

2. Patent application number *(The Patent Office will fill in this part)* — **0213286.8**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* —
The University of Edinburgh
Old College,
South Bridge,
EH8 9YL

Patents ADP number *(if you know it)* — 437767 7001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

SEE CONTINUATION SHEET

4. Title of the invention — PAR-2-ACTIVATING PEPTIDE DERIVATIVE AND PHARMACEUTICAL COMPOSITION USING THE SAME

5. Name of your agent *(if you have one)* — J.A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* —
14 South Square
Gray's Inn
London
WC1R 5JJ

Patents ADP number *(if you know it)* — 26001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) — Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	1 ✓
Description	24 ✓
Claim *(s)*	2 ✓
Abstract	1 ✓
Drawing *(s)*	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	1 ✓
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature J.A. Kemp & Co Date 10 June 2002

J.A. KEMP & CO.

12. Name and daytime telephone number of person to contact in the United Kingdom

CRESSWELL, Thomas Anthony
020 7405 3292

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

N.85898 TAC

CONTINUATION – PART 3 OF FORM 1/77

REQUEST FOR GRANT OF A PATENT

ADDITIONAL APPLICANTS

The University of Strathclyde
16 Richmond Street
Glasgow
G1 1XQ
United Kingdom.

4081667004

Kowa Company, Ltd.
6-29, Nishiki 3-chome
Naka-ku
Nagoya-shi
Aichi 460-0003
Japan.

8288367001

PAR-2-ACTIVATING PEPTIDE DERIVATIVE AND PHARMACEUTICAL COMPOSITION USING THE SAME

Field of the Invention

The present invention relates to a peptide derivative with a PAR-2-activating action or a salt thereof, and a pharmaceutical composition comprising the same as the effective ingredient. More specifically, the invention relates to a PAR-2-activating agent. Still more specifically, the invention relates to a prophylactic and therapeutic agent of the decrease of lacrimal fluid secretion, the decrease of saliva secretion or gastrointestinal diseases.

Background of the Invention

PAR (Protease-activated receptor)-2 is one the of the PARs belonging to 7-transmembrane G-protein-coupled receptor family.

Four types of PARs namely PAR-1, PAR-2, PAR-3 and PAR-4 have been cloned so far and these belong to a receptor family mediating the actions of serine proteases such as thrombin and trypsin in various cells. The functions of PAR-1, PAR-2, PAR-3 and PAR-4 have been elucidated as receptors involved in platelet aggregation via thrombin. Structurally or from the standpoint of activation mechanism, PAR-2 has numerous properties common

to other PARs. Functionally, however, PAR-2 is never activated by thrombin but is activated by trypsin and tryptase.

A specific site in the N-terminal amino acid sequences of these PARs is cleaved by thrombin or such proteases. Peptide fragments generated by the cleavage bind to the binding site of receptors thereof of themselves to activate the receptors. The amino acid sequences activating the PARs are summarized and expressed according to the one character amino acid expression as below.

PAR-1:	SFLLRN-NH_2	(human)
PAR-2:	SLIGKV-NH_2	(human)
	SLIGRL-NH_2	(mouse)
PAR-3:	None	
PAR-4:	GYPGQV	(human)
	GYPGKF	(mouse)

PAR-1, PAR-2 and PAR-4 can be activated non-enzymatically by exogenously-added peptides with the amino acid sequences of the peptide fragments generated via the cleavage but PAR-3 cannot be activated by such exogenously-added peptides. Recent research works have verified that mouse PAR-3 is the cofactor of PAR-4 since PAR-3 per se is never activated but is activated in the co-presence of PAR-4 (Nature, 404, 609-613 (2000)).

Cloning of PAR-2 was done in 1994 by Nystedt (Proc. Natl.

Acad. Sci. USA, 91, 9208-9212 (1994)). It is known that PAR-2 is activated by tissue factors Factor VIIa and Factor Xa, acrosin as one of sperm proteases, trypsin-like serine protease identified in rat brain, trypsin, tryptase and synthetic peptides of similar sequences as those of PAR-2 ligands (Pharmacological Rev, 53, 245-282, 2001; Br. J. Pharmacol. 1998, 123, 1434-1440).

Furthermore, some PAR-2-activating agents have been reported, which have higher activity than the activity of a partial PAR-2 amino acid sequence (SLIGKV) activating human PAR-2 and include trans-cinnamoyl-LIGRL-O-NH_2 found by Hollenberg et al. (Br. J. Pharmacol. 1998, 123, 1434-1440) (PNAS, 95, 7766-7771 (1998); BJP, 125, 1445-1454 (1998)).

A report also tells that pharmaceutical agents containing a PAR-2-activating agent as the effective ingredient are useful for the prophylaxis and therapeutic treatment of the decrease of saliva secretion, the decrease of lacrimal fluid secretion or gastrointestinal diseases (Japanese Patent Laid-open Nos. 064203/2001, 181208/2001 and 233790/2001).

However, compounds already reported as PAR-2 agonists are not satisfactory in terms of biological properties, physico-chemical properties, and ready synthesis. Therefore, further examinations have been needed for the development of such PAR-2 agonists into pharmaceutical products.

Summary of the Invention

The invention provides a compound with an excellent PAR-2-activating action and a pharmaceutical composition comprising the same as the effective ingredient.

In such circumstances, the present inventors have made intensive investigations. Consequently, the inventors have found that a peptide derivative represented by the general formula (I) has a higher activity than the activity of the partial PAR-2 amino acid sequence (SLIGKV) activating human PAR-2 and is useful as a pharmaceutical agent for the prophylaxis and therapeutic treatment of the decrease of lacrimal fluid secretion, the decrease of saliva secretion or gastrointestinal diseases. Thus, the invention has been achieved.

More specifically, the invention relates to a peptide derivative represented by the general formula (I) or a salt thereof:

$$Z\text{-}(CH_2)_n\text{-CO-NH-Leu-Ile-Gly-}AA_1\text{-}AA_2\text{-COOH} \qquad (I)$$

wherein Z represents an aryl group which may or may not have a substituent or a heteroaryl group which may or may not have a substituent; n represents 0, 1 or 2; and AA_1-AA_2 represents Lys-Val or Arg-Leu.

Additionally, the invention relates to a pharmaceutical composition comprising a peptide derivative represented by the general formula (I) or a salt thereof, and a pharmaceutically

acceptable carrier thereof.

Preferred Embodiments of the Invention

The peptide derivative of the invention is represented by the general formula (I), where the aryl group as Z is a carbon cyclic group of mono-ring type, multi-ring type or condensed ring type, with 6 to 30 carbon atoms, preferably 6 to 14 carbon atoms, specifically including for example phenyl group and naphthyl group, preferably. The heteroaryl group as Z is a hetero-cyclic group of 5- to 7-membered mono-ring type, multi-ring type or condensed ring type, the group containing at least one to 3 nitrogen atoms, oxygen atoms or sulfur atoms within the ring and specifically including for example furyl group, thienyl group, pyridyl group or quinolyl group, preferably.

The aryl group or heteroaryl group as Z may or may not have a substituent, which includes but is not limited to any aryl group or heteroaryl group with no adverse effects on the activity of the inventive peptide derivative, specifically including for example a halogen atom, a lower alkyl group, a lower alkoxyl group, phenyl group, a phenyl-lower alkyl group, nitro group, amino group, hydroxyl group, and carboxyl group. The halogen atom includes for example chlorine atom, fluorine atom, and bromine atom. The lower alkyl group is preferably a linear or branched lower alkyl group with one to 15 carbon

atoms, preferably one to 6 carbon atoms, which includes for example methyl group and ethyl group. The lower alkoxyl group preferably includes a linear or branched lower alkoxyl group with one to 15 carbon atoms, preferably one to 6 carbon atoms, which includes for example methoxyl group and ethoxyl group. The lower alkyl group in the phenyl-lower alkyl group includes alkylene groups including the lower alkyl group, for example methylene group and ethylene group.

Substituents for these lower alkyl group, lower alkoxyl group, phenyl group, and phenyl-lower alkyl group may additionally be substituted with a halogen atom and the like.

The group Z in the general formula (I) in accordance with the invention includes for example substituted or unsubstituted phenyl group, naphthyl group, furyl group, thienyl group, pyridyl group and quinolyl group, specifically including for example phenyl group, 4-methoxyphenyl group, 3-methoxyphenyl group, 2-methoxyphenyl group, 2,4-dimethoxyphenyl group, 3,5-dimethoxyphenyl group, 4-phenethylphenyl group, 3-phenethylphenyl group, 2-phenethylphenyl group, 4-nitrophenyl group, 3-nitrophenyl group, 2-nitrophenyl group, 2,4-dinitrophenyl group, 3,4-dinitrophenyl group, 4-methylphenyl group, 3-methylphenyl group, 2-methylphenyl group, 2,4-dimethylphenyl group, 3,5-dimethylphenyl group, 4-fluorophenyl group, 3-fluorophenyl group, 2-fluorophenyl group, 2,4-difluorophenyl group, 3,5-difluorophenyl group,

2,4,5-trifluorophenyl group, 4-phenylphenyl group, 3-phenylphenyl group, 2-phenylphenyl group, 2-furyl group, 3-furyl group, 5-methoxy-2-furyl group, 5-methyl-2-furyl group, 1-naphthyl group, 2-naphthyl group, 4-methoxy-1-naphthyl group, 4-methyl-1-naphthyl group, 4-methoxy-2-naphthyl group, 4-methyl-2-naphtyl group, 4-pyridyl group, 2-pyridyl group, 3-pyridyl group, 2-methyl-4-pyridyl group, 4-methyl-2-pyridyl group, 2-thienyl group, 3-thienyl group, 3-methyl-2-thienyl group, 4-methyl-2-thienyl group, 4-methyl-3-thienyl group, 6-quinolyl group, 7-quinolyl group, 8-quinolyl group, 4-quinolyl group, 4-methyl-6-quinolyl group and the like.

In the general formula (I), in accordance with the invention, n represents 0, 1 or 2 and the group with the inferior letter "n" is bound to the group Z. When n is 0, the group Z is directly bound to carbonyl group; when n is 1, the group z is bound through methylene group to carbonyl group; and when n is 2, the group Z is bound through ethylene group to carbonyl group.

In accordance with the invention, AA_1-AA_2 in the general formula (I) represents two types of amino acids bound together. The amino acid AA_1 is preferably Lys or Arg, while AA_2 is preferably Val or Leu. AA_1 and AA_2 are bound together in the sequence AA_1-AA_2 along the N-terminal to C-terminal direction. Preferable AA_1-AA_2 includes Lys-Val or Arg-Leu.

The C terminus of the peptide derivative represented by the general formula (I) of the invention is preferably free,

but may satisfactorily be esterified or amidated or may form a salt for formulation. The salt of the peptide derivative represented by the general formula (I) in accordance with the invention can be selected from pharmaceutically acceptable salts. For example, the salt can be a salt with an inorganic base or an organic base. The inorganic salt includes for example a salt with an alkali metal such as sodium or potassium, an alkali earth metal such as calcium or magnesium, aluminum salt and ammonium salt. The organic salt includes for example a salt with primary amines such as methylamine, ethylamine and ethanolamine, secondary amines such as diethylamine, diethanolamine, dicyclohexylamine, and N,N′-dibenzylethylenediamine, and tertiary amines such as trimethylamine, triethylamine, pyridine, picoline, and triethanolamine. Additionally, the salt may be a salt with basic amino acids such as arginine, lysine and ornithine.

PAR-2 activation with the peptide derivative represented by the general formula (I) in accordance with the invention can be tested by various known methods. For example, the Hollenberg's method (Hollenberg, M.D., et al., *Can. J. Physiol. Pharmacol.*, 75, 832-841 (1997)), the Kawabata's method (Kawabata, A., et al., *J. Pharmacol. Exp. Ther.*, 288, 358-370 (1999)) and the Plevin's method (Plevin, R. et al., Vasopressin stimulated [^{3}H]inositol phosphate and [^{3}H]-phosphatidylbutanol accumulation in A10 vascular smooth muscle cells Br., J.

Pharmacol. 107, 109-115)(1992)) may be used for the test.

The inventors tested the PAR-2 activation potency according to the Plevin's method. In brief, that is assay of the accumulation of [^{3}H]inositol Phosphate ([^{3}H]IP) in cells expressing human PAR-2. In this assay, cells were labelled with myo-[^{3}H]-inositol and stimulated with agonists in the presence of lithium, an inhibitor of inositol monophosphatase. The agonist-mediated accumulation of [^{3}H]-inositol phosphates (mainly IP_1) was quantified using ion-exchange chromatography.

The method is illustrated more specifically as follows.

NCTC2544 cells stably expressing PAR-2 were seeded in 24-well plates and subconfluent cells were pre-labelled with 1μCi/ml [2-^{3}H] myo-inositol in serum free medium (M199). Fifteen minutes prior to agonist stimulation, LiCl was added at a final concentration of 10mM. Then cells were stimulated with various concentrations of test compounds. After 1hr for stimulation, cells were washed twice with 2ml ice-cold PBS and the lipid fraction was extracted with 500μl methanol. The cells were detached from each well using a cell-scraper and collected into a 5ml-scintillation vial. The residual cell extract was rinsed with another 500μl of methanol and combined with the initial sample. 500μl of chloroform was added to give a 2:1 methanol: chloroform and then the sample mixed well by vortexing and left for 60min. Subsequently, another 500μl of chloroform and 800μl of H_2O were added (final contents: 1ml of methanol; 1ml of

chloroform; and 0.8ml of H_2O) and mixed again. When the organic phase had clearly separated, 1ml of the aqueous phase (upper layer) was transferred to another vial containing 500μl of pre-washed anion exchange resin (AG1-X, formate form). Following two-washes with 2.5ml water, the resin was also washed with 2.5ml of washing buffer [5mM sodium tetraborate, 60mM ammonium formate in water] to remove [^{3}H]-glycerophosphoinositol (GPI). After washing again with 2.5ml of water, total phosphoinositides ([^{3}H]-IP_{1-4}) were eluted by addition of 1ml elution buffer [1M ammonium formate, 0.1M formic acid in water]. Supernatant containing labelled inositol phosphates was transferred into an another scintillation vial and [^{3}H] radioactivity was measured by a scintillation counter (Packard) followed by the addition of 4ml of scintillation cocktail (Hi-Safe3, Wallac).

SLIGKV-OH as a known PAR-2 activation peptide was used as a comparative compound, and the results are shown below in Table 1.

Table 1

Compound (Example No.)	MW	Agonist Activity (EC50, μM)
SLIGKV	615.0	50
Example 1	663.4	30.49 +/- 3.0
Example 3	661.64	6.18 +/- 1.9
Example 5	647.52	25.99 +/- 8.7
Example 6	623.57	8.33 +/- 1.3
Example 7	678.50	24.30 +/- 6.3

Consequently, it was shown that phenethylcarbonyl derivative shown below in Example 3 (the group Z-$(CH_2)_n$- is phenyl group in the general formula (I)) was at an EC_{50} value about 1/8-fold that of the comparative peptide and that 2-furylcarbonyl derivative shown below in Example 6 (the group Z-$(CH_2)_n$- is 2-furyl group in the general formula (I)) was at an EC_{50} value about 1/6-fold that of the comparative peptide. Additionally, other peptide derivatives of the invention had activation potencies about 2-fold that of the comparative peptide.

The peptide derivative of the invention can be prepared by various other synthetic methods of related art or methods according to such methods, with no specific limitation. For example, the peptide derivative can be prepared at the following reaction method.

Following the amino acid sequence of an intended peptide by general peptide-bond-forming reactions by liquid phase process or solid phase process, the peptide derivative of the invention can be prepared through a route of sequentially binding together individual amino acids one by one or a route of binding together individual fragments of several amino acids preliminarily synthesized. For peptide-bond formation, various known methods can be applied, namely a method for converting the C-terminal carboxyl group of an amino acid or a peptide into a reactive functional group, or a method for using general condensation agents. Examples of the reactive derivatives of amino acid or peptide include halogenated acids such as acid chloride, azidated acids, symmetric acid anhydrides, mix acid anhydrides with pivalic acid, and activated esters such as p-nitrophenyl ester. Examples of the condensing agent include 1,3-dicyclohexylcarbodiimide, 1-cyclohexyl-3-morpholinoethylcarbodiimide, 1-(3-diaminopropyl)-3-ethylcarbodiimide, 1,1'-carbonyldiimidazole, diethylphosphate cyanide, diphenylphosphoryl azide, bis(2-oxo-3-oxazolidine)phosphinyl chloride, and 2-chloro-1-methylpyridinium iodide.

For these reactions, if necessary, an appropriate base or an appropriate solvent may be used. The base includes for example organic bases such as pyridine, triethylamine, and diisopropylethylamine; or inorganic bases such as sodium

carbonate and sodium hydrogen carbonate. The solvent includes for example dimethylformamide, tetrahydrofuran, dioxane, acetonitrile, methylene chloride and dichloroethane.

When using a condensing agent such as 1,3-dichlorohexylcarbodiimide, appropriate activating agents such as 1-hydroxybenzotriazole and N-hydroxysuccinimide can accelerate the reaction velocity when added, effectively, to suppress racemization. Various reagents listed herein may be used in forms bound to resins such as polystyrene, so as to simplify the isolation procedure of the peptide synthetically prepared.

During the peptide-bond-forming reaction, further, functional groups that should never be involved in the reaction because of their potential side effects are preferably protected. The protective group of the amino group includes for example benzyloxycarbonyl, t-butoxycarbonyl, allyloxycarbonyl, 9-fluorenylmethoxycarbonyl, p-methoxybenzyloxycarbonyl, 3-nitro-2-pyridinesulphenyl, trifluoroacetyl, phthaloyl, and formyl. The protective group of the carboxyl group includes for example methyl ester, ethyl ester, and benzyl ester. Additionally, the protective group of the guanidyl group in arginine includes for example p-toluenesulfonyl, nitro, benzyloxycarbonyl, and 4-methoxy-2,3,6-trimethylbenzenesulfonyl.

Depending on the property of each of these protective groups, the protective groups can be eliminated by acid treatment, base

treatment, reduction, hydrolysis and the like. Acids including for example hydrogen chloride, hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, trimethylsilane bromide, trimethylsilyltrifluoromethanesulfonate, tetrafluoroboric acid, and boron bromide can be used as the acid. The base includes for example piperidine, pyrrolidine, triethylamine, and diisopropylethylamine. Additionally, the reduction conditions include the use of sodium/liquid ammonia, palladium catalyst/hydrogen, palladium catalyst/formic acid and the like. For the hydrolysis, lithium hydroxide and sodium hydroxide can be used.

The N-terminal acyl group in the peptide derivative of the invention may be modified into N-acylated leucine via the reaction between the corresponding carboxylic acid or a reactive derivative and leucine of which the functional groups that should not be involved in the reaction are protected. The resulting N-acylated leucine may be used for the peptide synthesis. Otherwise, after the synthesis of the object peptide or during the synthetic process, the amino group in leucine is similarly acylated. The conditions for the peptide-bond-forming reaction are applicable to the reaction conditions then.

The compound of the invention as recovered by the method can be purified if necessary by general methods, for example gel chromatography, partition chromatography, ion exchange

chromatography, affinity chromatography, countercurrent distribution chromatography, high performance liquid chromatography with various carriers, and recrystallization.

Due to the PAR-2-activating action, the peptide derivative represented by the general formula (I) in accordance with the invention has an action to promote lacrimal fluid secretion, an action to promote saliva secretion, an action to suppress gastric juice secretion, an action to promote mucus secretion, and an action to protect mucosal membrane. Thus, the peptide derivative is useful as a prophylactic and therapeutic agent of dysfunction of masticatory, dysphagia, dysgeusia, ozostomia, intra-oral cavity dysphoria, intra-oral cavity infections, intra-oral cavity inflammations, dry eye, ectocornea detachment, keratitis, corneal ulcer, conjunctivitis, stomach ulcer, duodenal ulcer, gastritis, diarrhea, or enteritis.

The pharmaceutical composition of the invention contains the peptide derivative represented by the general formula (I) of the invention or a salt thereof, and a pharmaceutically acceptable carrier thereof. The pharmaceutically acceptable carrier includes various additives for general pharmaceutical use. For example, the pharmaceutically acceptable carrier includes solubilizers, excipients, binders and diluents. The pharmaceutical composition of the invention can be formulated into various dosage forms, using these carriers. The

pharmaceutical composition of the invention can be formulated into dosage forms, for example, tablets, capsules, granules, powders, lotions, ointments, injections and suppositories.

Additionally, the pharmaceutical composition of the invention can be prepared by known methods. For the formulation of oral dosage forms, for example, an appropriate combination of the following additives may be used for formulation; solubilizers such as gum tragacanth, gum arabic, sucrose ester, lecithin, olive oil, soybean oil, and PEG400; excipients such as starch, mannitol, and lactose; binders such as sodium carboxymethylcellulose, and hydroxypropyl cellulose; disintegrators such as crystal cellulose and carboxymethylcellulose calcium; lubricants such as talk and magnesium stearate; and fluidity-enhancing agents such as light anhydrous silicic acid.

The pharmaceutical composition of the invention can be administered orally or parenterally. For example, the pharmaceutical composition can be administered by intravenous injection, trans-mucosal dosing, trans-dermal dosing, intra-muscular dosing, subcutaneous dosing and intra-rectal dosing.

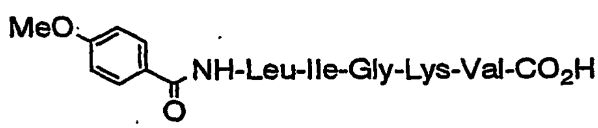
The dose of the pharmaceutical composition of the invention varies, depending on the body weight, age, sex and symptom of a patient and the like. On a basis of the effective ingredient peptide derivative represented by the general formula (I) of

the invention, the pharmaceutical composition is generally administered at a dose of the peptide derivative of 0.01 to 1,000 mg, preferably 0.1 to 100 mg in one portion or three-dividend portions per day per adult.

Examples

The invention will now be described more specifically in the following Examples. But the technical scope of the invention is not limited to these Examples.

Example 1 (Preparation for



)

(1) Loading of resin:

Fmoc-Val-OH (6 eq.) was dissolved in DMF and 1,3-diisopropylcarbodiimide (3 eq.) was added. The mixture was sonicated for 10min and added to Wang-resin (0.1 mmol), which was swollen in DMF and catalytic amount of DMAP (30 mg) added. The resin mixture was then sonicated for 2h at room temperature. Loading was 0.37 mmol/g.

(2) Peptide chain assembly:

The peptide chain was synthesized on an ABI430 peptide synthesizer. 1,3-diisopropylcarbodiimide/HOCt were used as

coupling reagents. Single coupling cycles were applied and the deprotection of each cycle was monitored by UV at 302nm.

(3) Coupling of 4-methoxybenzoic acid to the peptide:

After the deprotection of the last amino acid, the resin was removed from the synthesizer, washed with DMF/dichloromethane, and dried. 4-methoxybenzoic acid (5 eq.) was dissolved in dichloromethane and triphosgene (6 eq.) was added. The mixture was stirred for 30 min at 0 °C and added to the peptide resin without further purification. N,N-Diisopropylethylamine (10 eq.) was added to the mixture and it was sonicated for 1 h at room temperature. The reaction mixture was filtered off and the resin was washed with DMF/dichloromethane and dried.

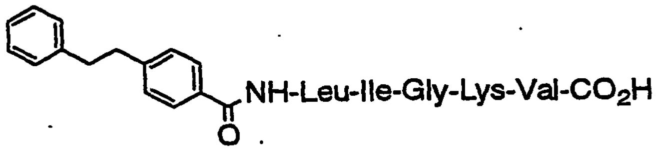
(4) Cleavage and purification:

The peptoid was cleaved with 90% TFA/H_2O and purified by HPLC [Column: Vydac C18 (250 x 22 mm); Solvent system: A(0.1% TFA/H_2O), B(0.1% TFA/CH_3CN), 10-50% B over 30 min; Flow rate: 5 mL/min; Detection: UV at 214 nm]. Analytical HPLC [Column: Vydac C18 (250 x 10 mm); Solvent system: A(0.1% TFA/H_2O), B(0.1% TFA/CH_3CN), 10-90% B over 30 min; Flow rate: 1 mL/min; Detection: UV at 214 nm] was used to check the purity and electrospray MS was used for identification. The title compound was in >95% purity according to HPLC.

Retention time: 19.8 min

MS (m/z): 663.4 (required 662.83)

Example 2 (Preparation for



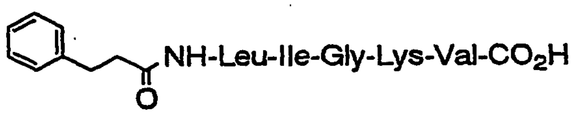
)

The title compound was prepared in the same manner as in Example 1 except that 4-phenethylbenzoic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 23.4 min

MS (m/z): 737.5 (required 736.96)

Example 3 (Preparation for



)

The title compound was prepared in the same manner as in Example 1 except that 3-phenylpropionic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 25.1 min

MS (m/z): 661.6 (required 660.86)

Example 4 (Preparation for

)

The title compound was prepared in the same manner as in Example 1 except that 2,4-dinitrobenzoic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 19.7 min

MS (m/z): 723.5 (required 722.80)

Example 5 (Preparation for

)

The title compound was prepared in the same manner as in Example 1 except that 4-methylbenzoic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 20.6 min

MS (m/z): 647.5 (required 646.83)

Example 6 (Preparation for

)

The title compound was prepared in the same manner as in Example 1 except that 2-furancarboxylic acid was used instead

of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 24.2 min

MS (m/z): 623.5 (required 622.76)

Example 7 (Preparation for

)

The title compound was prepared in the same manner as in Example 1 except that 2-(4-methoxyphenyl)acetic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 23.7 min

MS (m/z): 678.5 (required 676.86)

Example 8 (Preparation for

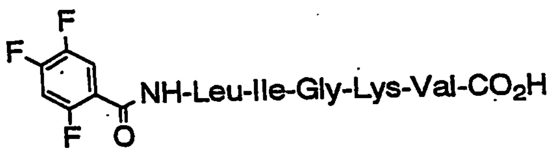
)

The title compound was prepared in the same manner as in Example 1 except that 2-naphthalenecarboxylic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 26.0 min

MS (m/z): 683.2 (required 682.86)

Example 9 (Preparation for



)

The title compound was prepared in the same manner as in Example 1 except that 2,4,5-trifluorobenzoic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 20.6 min

MS (m/z): 687.9 (required 686.78)

Example 10 (Preparation for

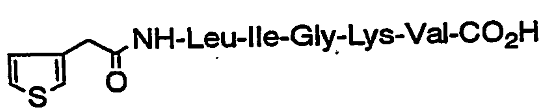
)

The title compound was prepared in the same manner as in Example 1 except that 4-pyridinecarboxylic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 14.4 min

MS (m/z): 634.5 (required 633.79)

Example 11 (Preparation for



)

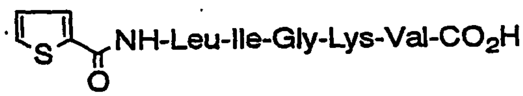
The title compound was prepared in the same manner as in

Example 1 except that 2-(3-thienyl)acetic acid was used instead of 4-metoxy benzoicacid as the acid to be coupled.

Retention time: 19.0 min

MS (m/z): 653.6 (required 652.86)

Example 12 (Preparation for



)

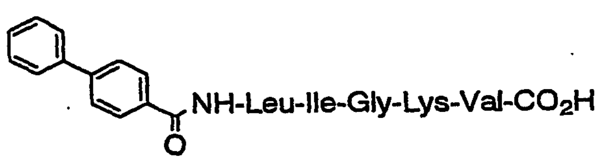
The title compound was prepared in the same manner as in Example 1 except that 2-thiophenecarboxylic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 21.0 min

MS (m/z): 639.6 (required 638.82)

Example 13 (Preparation for

)

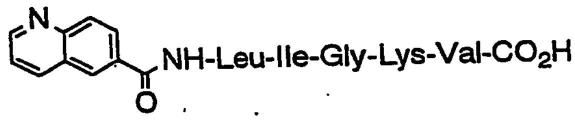


The title compound was prepared in the same manner as in Example 1 except that 4-phenylbenzoic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 23.2 min

MS (m/z): 709.4 (required 708.91)

Example 14 (Preparation for



)

The title compound was prepared in the same manner as in Example 1 except that 6-quinolinecarboxylic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

Retention time: 17.3 min

MS (m/z): 684.4 (required 683.87)

Example 15 (Preparation for

)

The title compound was prepared in the same manner as in Example 1 except that 3-quinolinecarboxylic acid was used instead of 4-metoxybenzoic acid as the acid to be coupled.

MS (m/z): 683.5 (required 683.87).

What is claimed is:

1. A peptide derivative represented by the general formula (I) or a salt thereof:

$$Z-(CH_2)_n-CO-NH-Leu-Ile-Gly-AA_1-AA_2-COOH \quad (I)$$

wherein Z represents an aryl group which may or may not have a substituent or a heteroaryl group which may or may not have a substituent; n represents 0, 1 or 2; and AA_1-AA_2 represents Lys-Val or Arg-Leu.

2. The peptide derivative or a salt thereof according to claim 1, where Z represents an aryl group which may or may not have a halogen atom, a lower alkyl group, a lower alkoxyl group, phenyl group, a phenyl-lower alkyl group or nitro group as the substituent or a heteroaryl group which may or may not have a halogen atom, a lower alkyl group, a lower alkoxyl group, phenyl group, a phenyl-lower alkyl group or nitro group as the substituent.

3. The peptide derivative or a salt thereof according to claim 1 or 2, where the aryl group is phenyl group or naphthyl group; and the heteroaryl group is furyl group, thienyl group, pyridyl group or quinolyl group.

4. A pharmaceutical composition comprising a peptide derivative or a salt thereof according to any one of claims 1 to 3, and a pharmaceutically acceptable carrier thereof.

5. The pharmaceutical composition according to claim 4, which is a PAR-2-activating agent.

6. The pharmaceutical composition according to claim 4 or 5, which is a prophylactic and therapeutic agent of the decrease of saliva secretion, the decrease of lacrimal fluid secretion or gastrointestinal disorders.

7. The pharmaceutical composition according to any one of claims 4 to 6, where the pharmaceutical composition is a prophylactic and therapeutic agent of dysfunction of masticatory, dysphagia, dysgeusia (taste disorder), ozostomia, intra-oral cavity dysphoria, intra-oral cavity infections, intra-oral cavity inflammations, dry eye, ectocornea detachment, keratitis, corneal ulcer, conjunctivitis, stomach ulcer, duodenal ulcer, gastritis, diarrhea, or enteritis.

Abstract

The present invention relates to a peptide derivative represented by the general formula (I) or a salt thereof:

$$Z\text{-}(CH_2)_n\text{-CO-NH-Leu-Ile-Gly-}AA_1\text{-}AA_2\text{-COOH} \qquad (I)$$

wherein Z represents an aryl group which may or may not have a substituent or a heteroaryl group which may or may not have a substituent; n represents 0, 1 or 2; and AA_1-AA_2 represents Lys-Val or Arg-Leu, and relates to a pharmaceutical composition comprising a peptide derivative represented by the general formula (I) or a salt thereof, and a pharmaceutically acceptable carrier thereof.